

Preclinical Study of Ketoconazole Oretentive Medicated Jelly

Anand Ambekar¹, Ajaykartik², Vinay B. Shinde³ Pratima.S¹ and Purushotham Rao.K*³

¹Department of Pathology,

MR Medical College and General Hospital, Gulbarga, K.S,

²Panineeya Institute of Dental Sciences and Research Center,

Hyderabad, T.S.

³HKESs Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences,
Gulbarga. K.S

*Corresponding author e-mail: kprao369@rediffmail.com

ABSTRACT

Objective: Convenience of administration and patient compliance are gaining significant importance in the design of dosage forms. Difficulty in swallowing (dysphasia) is common among all age groups, especially in elderly and pediatrics. Ketoconazole was formulated as orotretentive jelly for the treatment of oral candidiasis. There are dosage forms like syrups, tablets in the market but still there is a need for new dosage form which acts effectively and locally. The jellies can provide an attractive alternative formulation in the treatment of oral candidiasis. So the present investigation aims to design, prepare and evaluate ketoconazole jellies using polymers such as xanthan gum, sodium carboxy methyl cellulose with different concentrations. The benefits of these prepared jellies are increased bioavailability, reduction in gastric irritation by-passing first pass metabolism.

Methods: The sucrose based jellies were prepared by heating and congealing method. Preformulation studies, organoleptic, physical characteristics, drug content, pH, spreadability, syneresis, *in vitro* dissolution testing, drug release kinetics and stability studies were conducted.

Results: The prepared formulations are free from gritty particles. All the formulations were tested for drug excipient interactions subjecting to IR Spectral analysis. *In vitro* drug dissolution studies showed 95.12% for K₁, 90.66% for K₂ and 95.22% for K₃ in 30 minutes. Among the 7 formulations, formulation K₃ containing 5% Sodium carboxy methyl cellulose was found to be promising. Short-term stability studies on the promising and other formulations indicated that there were no significant changes in the drug content and *in vitro* dissolution characteristics. IR spectroscopic studies indicated that there were no drug-excipient interactions. Anti-fungal studies revealed that there is no change in the molecular activity of the drug. Results of *in vivo* studies indicated compatible drug delivery.

Conclusions: The prepared jellies of ketoconazole could stay in the mouth for a longer period of time, which indicates a potential use of jellies of ketoconazole for treating oral candidiasis.

Keywords: Ketoconazole; Jellies; Xanthan gum; Sodium carboxy methyl cellulose.

INTRODUCTION

Oral candidiasis is one of the common fungal infections, affecting the oral mucosa. These lesions are caused by the yeast *Candida albicans*. Candidiasis is defined as an infection caused by a fungi of the genus *Candida*, and the term oral candidiasis is only used when describing a clinically visible lesion in the oral cavity.¹ The conventional formulations for the local delivery of drugs to the oral cavity are the mouth paints, rinses, troches, creams and suspensions.^{2,3} One way to improve the efficacy in eradicating the infection is to deliver the antifungal locally in the oral cavity. Better stability and longer residence time will allow more of the antifungal to penetrate through the oral mucous layer to act on *Candida* species for longer duration of time. Therefore some researchers had prepared and reported new formulation such as gels, mucoadhesive tablets, pH sensitive excipients composition mucoadhesive microspheres, which were able to reside in oral cavity for an extended period for more effective candidiasis eradication.^{4,5} The present investigation is designed to improve patient compliance. Advantages of the Ketoconazole jellies as dosage forms include increase in bioavailability, reduction in dose size, and in gastric irritation, bypass first pass metabolism. The present work is aimed at preparing a formulation of Ketoconazole jellies, for relief of oral candidiasis.

MATERIALS AND METHODS

Ketoconazole was received as a gift sample from M/s Alkem Laboratories Pvt. Ltd., Mumbai. Carboxy Methyl cellulose sodium salt was obtained from Sol Fine Chemicals Pvt. Ltd., Mumbai. Xanthan gum

was obtained from Local market. Sucrose was brought from SD Fine Chemicals, Mumbai. Citric acid was received from CDH Pvt. Ltd., Mumbai. All other chemicals and solvents used are of analytical grade and used as procured.

PREPARATION OF MEDICATED JELLIES⁶

All the formulations were prepared using freshly boiled and cooled distilled water as per the composition listed in Table 1. Ketoconazole jellies were prepared by heating and congealing method. Syrupy base was prepared in a copper vessel dissolving the required amounts of sugar in water on heating and stirring at 80°C for about 90 min. accurately weighed polymer powder was dispersed in 10 mL of purified water maintained at 90°C throughout preparation. The dispersion was stirred using a magnetic stirrer (2MLH, Remi Equipment Pvt. Ltd., Mumbai, India) for 20 min to facilitate hydration of gelling agent. Ketoconazole was taken in another beaker and solubilized using alcohol. Then simple syrup was added to it under continuous stirring. Then citric acid and preservatives were added under continuous stirring. Color and flavor was added to this under continuous stirring at 60°C. The final weight was adjusted with purified water, mixed, transferred to polyethylene molds, sealed and allowed to cool at room temperature (25°C ± 5°C) to form a jelly like texture. After the jelly is set it is wrapped in to the gelatin paper and stored in dry place.

CHARACTERISATION OF PREPARED KETOCONAZOLE JELLIES

Physical observation⁷

The prepared jellies were observed visually for clarity, odor, texture and presence of any particles. The texture was evaluated in terms of stickiness and grittiness by mild rubbing the jelly between two fingers.

Weight variation⁸

The average weight of ten jellies was taken to determine weight variation. The jellies were taken out of the molds in a beaker and weighed individually, pooled and mixed.

Determination of pH^{9,10}

The pH of the formulation influences the taste and stability of oral jellies. The pH of prepared jellies was measured using a digital pH meter (LI 120, Elico Ltd., Hyderabad, India) at room temperature ($25^{\circ}\text{C} \pm 5^{\circ}\text{C}$). For this purpose, 0.5 g of jelly was dispersed in 50 mL of distilled water to make a 1% solution, and the pH was noted.

Syneresis¹¹

Gels experience syneresis or deswelling due to the release of liquid, resulting in shrinkage of gels and reduce quality. Syneresis is the contraction of the gel upon storage and separation of water from the gel. It is more pronounced in the gels, where lower concentration of gelling agent is employed. All the jellies were observed for signs of syneresis at room temp ($25^{\circ}\text{C} \pm 5^{\circ}\text{C}$). The formulations showing signs of syneresis were rejected and not considered for further studies.

DRUG-EXCIPIENT COMPATIBILITY STUDIES¹²

The drug and excipients were mixed together in 1:1 ratio and placed in borosilicate colored glass vials. These vials

were sealed and placed in an oven maintained at 40°C and 75% RH. The samples were observed after 15, 30 and 45 days for any color change or lump formation. Fourier transforms infrared (FTIR) spectra of the pure drug and its mixtures of gelling agents were measured by preparing dispersion in dry KBr using attenuated total reflectance FTIR spectrophotometer (Bruker, UK). The absorption maxima in the spectra obtained were compared, and the presence of additional peaks corresponding to the functional groups was noted.

STABILITY STUDIES^{13,14}

A physically stable medicated oral jelly should retain its viscosity, color, clarity, taste, and odor throughout its shelf-life. The stability studies were performed at two temperatures i.e., 37°C and 45°C over a period of six months. Sufficient number of samples (10) were packed in amber colored screw capped bottles and kept in incubator maintained at 37°C . Samples were taken at intervals of 15 days for the drug content estimation.

IN VITRO DRUG DISSOLUTION STUDIES^{15,16}

USP XXIII Dissolution test apparatus was used by taking 100 ml of pH 6.8 buffer in 1000 ml dissolution flask and jelly was placed in it, rotating paddle at a speed of 150 rpm and temperature $37 \pm 1^{\circ}\text{C}$ was maintained. 5 ml aliquots were withdrawn at 01, 02, 03, 04, 05 and 06 minutes intervals, after each withdrawal of a sample an equal volume of dissolution medium was added to the dissolution flask. The filtered samples were diluted and analyzed spectrophotometrically at 274.0 nm.

ANTIFUNGAL ACTIVITY¹⁷

Microbiological studies were carried out to ascertain the antifungal activity of the prepared formulation as against the pure drug. Ketoconazole is known to possess superior antifungal activity against fungal infections. In present work, antifungal activity of Ketoconazole was tested by using the yeast *Candida albicans*, which is the most frequently encountered human fungal pathogen being responsible for a wide range of superficial infections. The prepared jellies were evaluated for *in-vitro* antifungal activity using standard Agar cup-plate method.

ORAL MUCOSAL IRRITANCY ASSESSMENT^{18,19}

Oral mucosal compatibility studies of prepared formulations prepared without drug were carried out in healthy human volunteers under the supervision of qualified physicians

By examining each volunteer's oral cavity using focus lens to notice any changes in tissues after the usage of formulations. Then photographic imaging of oral cavity of human volunteers were taken after subsequent application for 72 hrs i.e., at completion of study period and these images were compared to determine the difference with the images taken at 0 hour of study i.e., prior to first usage of formulation. Mucosal irritation was also evaluated by questioning the human volunteers at regular intervals of time about the feeling of itching or irritancy which appears to be highly subjective for the study. Finally the oral mucosal skin irritancy was evaluated for any changes like oral erythematic, inflammation, redness, hemorrhagic lesions or acute painful ulcers (canker sores).

RESULTS: CHARACTERISATION OF PREPARED CLOTRIMAZOLE JELLIES

Physical observation

Physical observation of jellies is important to justify the patient acceptance and compliance of the products. The observed parameters are summarized in Table 2. of all the formulations K₃ showed best results being transparent and slightly sticky with an acceptable consistency.

Weight variation

The weight variation was found between $4.95\% \pm 0.58\%$ and $5.79\% \pm 0.83\%$ in all prepared jelly formulations.

Determination of pH

The results of pH of prepared jelly formulations are summarized in Table 3. The pH of the formulation influences the taste and stability of oral jellies. The pH of the prepared formulations was found in the range of 6.32 ± 0.03 - 6.93 ± 0.04 which was slightly acidic. Sucrose may precipitate in the presence of citric acid on standing. Therefore, a minimum quantity of citric acid was added just to maintain the pH.

Syneresis

Syneresis was more pronounced in the formulations, where lower concentration of gelling agent was employed. It was observed after 24 h of jelly preparation. The formulations K₁ showed syneresis at room temperature ($25^{\circ}\text{C} \pm 5^{\circ}\text{C}$) (Table 3).

Stability studies

A physically stable medicated oral jelly should retain its viscosity, color, clarity, taste, and odor throughout its shelf-life. The samples were characterized for change in various parameters such as appearance, pH, viscosity, sugar crystallization, stiffness, syneresis and drug content at the end of 90 days. A freshly

made sample was used as a reference standard for subjective evaluations. Formulation K₃ showed best results.

***In vitro* dissolution testing**

The *in vitro* dissolution study was carried out to compare Ketoconazole release kinetics from the prepared jellies. The results are summarized in Figure 1. K₃ showed optimal results.

Antifungal studies

The anti-microbial study reveals that zone inhibition of various prepared formulations was found to be equal on comparison with the activity of pure drug. This indicates that there is no change in the molecular activity of the drug present in the formulations. The results are summarized in table 4.

Oromucosal compatibility tests

Results of *in vivo* studies in healthy human volunteers under the supervision of qualified team of physicians revealed that no redness or ulcer formation or any irritation on oral mucosa was observed. Hence, the formulations prepared were compatible to use as drug delivery.

CONCLUSION

It is found that sucrose based Medicated jellies will be ideal dosage forms for patients. These will have additional advantages of patient compliance, convenience and comfortness for efficient treatment including low dose, immediate onset of action, reduced dosage regimen and economic. The Physico-chemical characterization revealed that all the formulations were found to be shown acceptable weight variation, pH, viscosity, spreadability and syneresis. The drug content estimation showed uniform drug content in all the formulations. IR spectroscopic studies indicated that there

were no drug-excipients interactions. Addition of hydrophilic mucoadhesive polymers like xanthan gum yield good results to prolong dissolution time and the drug release in salivary pH conditions for a period of 30 minutes. The stability studies proved that the prepared Medicated jellies were found to be stable when stored at air tight containers or twist strips. Hence the present piece of investigation will be used for industry, research and development division. The anti-microbial study reveals that zone inhibition of various prepared formulations was found to be equal on comparison with the activity of pure drug. This indicates that there is no change in the molecular activity of the drug present in the formulations. Results of *in vivo* studies in healthy human volunteers under the supervision of qualified team of physicians revealed that no redness or ulcer formation or any irritation on oral mucosa was observed. Hence, the formulations prepared were compatible to use as drug delivery. The present work Medicated Jellies offer patient convenience, compliance and comfortness in application and transportation with effective treatment.

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Table 1. Working formulae to prepare Ketoconazole jellies

Sl. No	Ingredient (w/w)	K ₁	K ₂	K ₃
01	Drug	0.2	0.2	0.2
03	Xanthan gum	-	1.5	
04	Sodium carboxy methyl cellulose	-	-	5
05	Citric acid	1	1	1
06	Alcohol	5	5	5
07	Methyl paraben	0.18	0.18	0.18
08	Propyl paraben	0.02	0.02	0.02
09	Color	Q.S	Q.S	Q.S
10	Flavor	Q.S	Q.S	Q.S
11	Sucrose	66.7	66.7	66.7
12	Purified water	28.8	28.8	28.8
13	Total weight	100	100	100

* Each jelly contains 10 mg of Drug.

* Each jelly weighs 05 gms.

Table 2. Physicochemical parameters of prepared jellies

Formulations	Properties of jellies				
	Appearance	Consistency	Texture	pH of the jelly	Syneresis (Room temp: 25°C± 5°C)
K ₁	Transparent	Slightly liquid	Non-sticky	6.32±0.08	-
K ₂	Acceptable	Thick	Non-sticky	6.93±0.01	-
K ₃	Acceptable	Acceptable	Slightly sticky	6.81±0.02	-

* All values are in triplicates

* Each jelly contains 02 mg of Drug.

* Each jelly weighs 05 gms.

Table 3. Antimicrobial studies showing the comparative zone of inhibition of drug as pure and in formulation (K₃)

Formulation Code	Statistical Zone inhibition (mm) after 36 hrs			Mean± S.D
	Zone 1	Zone 2	Zone 3	
Pure Drug	23	23	24	23.33±0.57
K ₃	22	24	21	22.33±1.53

Table 4. Oral Mucosal Compatibility studies of prepared jellies formulations without Drug in Healthy Human Volunteers

Formulation Code	Human Volunteers	Before Application			After 24 hrs of Application			After 48 hrs of Application			After 72 hrs of Application		
		I	R	E	I	R	E	I	R	E	I	R	E
K ₁	Male-I	X	x	x	x	x	x	x	x	x	X	x	x
	Female-I	x	x	x	x	x	x	x	x	x	X	x	x

I- Skin Irritation, R- Redness, E- Erythema

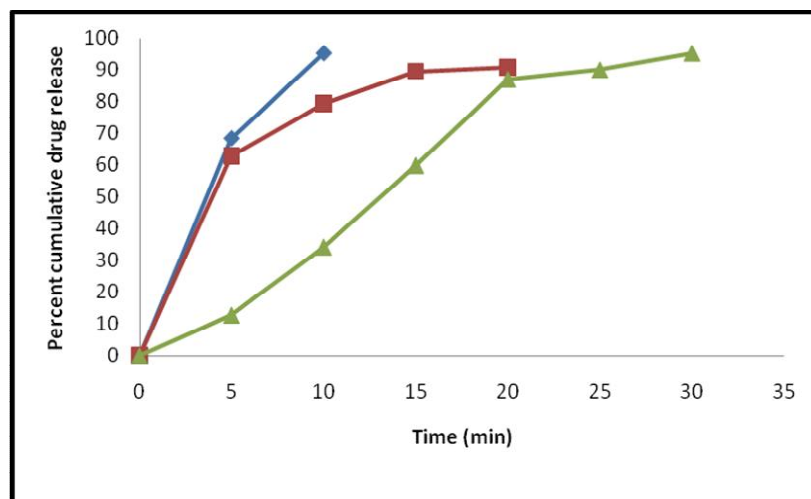


Fig. 1. *In vitro* studies of Ketoconazole medicated jellies

- * All values are in triplicates
- * Each jelly contains 02 mg of Drug.
- * Each jelly weighs 05 gms.

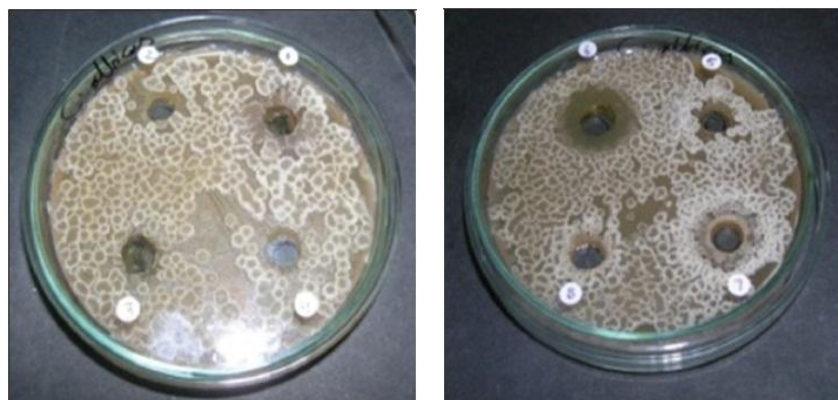


Fig. 2. Photographs of antimicrobial studies showing the comparative zone of inhibition of drug as pure and in formulation (K_3)



Male-I: Before Application



Male-I: After 72 hrs of Application



Female: Before Application



Female: After 72 hrs of Application

Figure 3. Oral Mucosal Compatibility studies of prepared jelly formulations without Drug in Healthy Human Volunteers